

type of food used, its source, feeding frequency and results of analysis (i.e., concentrations) for contaminants.

(7) Number of embryos hatched, number of healthy embryos, time to hatch, mortality of embryos and fry, measurements of growth (weight and length), incidence of pathological or histological effects and observations of other effects or clinical signs, number of healthy fish at end of test.

(8) Number of organisms that died or showed an effect in the control and the results of analysis for concentration(s) of any contaminant in the control(s) should mortality occur.

(9) Methods used for, and the results of (with standard deviation), all chemical analyses of water quality and test substance concentration, including validation studies and reagent blanks; the average and range of the test temperature(s).

(10) Anything unusual about the test, any deviation from these procedures, and any other relevant information.

(11) A description of any abnormal effects and the number of fish which were affected during each period between observations in each chamber, and the average concentration of test substance in each test chamber.

(12) Reference to the raw data location.

[50 FR 39321, Sept. 27, 1985, as amended at 52 FR 19064, May 20, 1987]

§ 797.1930 Mysid shrimp acute toxicity test.

(a) *Purpose.* This guideline is intended for use in developing data on the acute toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003, 15 U.S.C. 2601 *et seq.*). This guideline prescribes a test using mysid shrimp as test organisms to develop data on the acute toxicity of chemicals. The United States Environmental Protection Agency (EPA) will use data from these tests in assessing the hazard of a chemical to the aquatic environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and in part 792—*Good Laboratory Practice Standards* of this chap-

ter, apply to this test guideline. The following definitions also apply to this guideline.

(1) "Death" means the lack of reaction of a test organism to gentle prodding.

(2) "Flow-through" means a continuous or an intermittent passage of test solution or dilution water through a test chamber or a holding or acclimation tank, with no recycling.

(3) "LC₅₀" means that experimentally derived concentration of test substance that is calculated to kill 50 percent of a test population during continuous exposure over a specified period of time.

(4) "Loading" means the ratio of test organisms biomass (grams, wet weight) to the volume (liters) of test solution in a test chamber.

(5) "Retention chamber" means a structure within a flow-through test chamber which confines the test organisms, facilitating observation of test organisms and eliminating loss of organisms in outflow water.

(6) "Static system" means a test chamber in which the test solution is not renewed during the period of the test.

(c) *Test procedures*—(1) *Summary of the test.* In preparation for the test, test chambers are filled with appropriate volumes of dilution water. If a flow-through test is performed, the flow of dilution water through each chamber is adjusted to the rate desired. The test substance is introduced into each test chamber. In a flow-through test, the rate at which the test substance is added is adjusted to establish and maintain the desired concentration of test substance in each test chamber. The test is started by randomly introducing mysids acclimated in accordance with the test design into the test chambers. Mysids in the test chambers are observed periodically during the test, the dead mysids removed and the findings recorded. Dissolved oxygen concentration, pH, temperature, salinity, the concentration of test substance, and other water quality characteristics are measured at specified intervals in test chambers. Data collected during the test are used to develop concentration-response curves and LC₅₀ values for the test substance.

(2) [Reserved]

(3) *Range-finding test.* (i) A range-finding test should be conducted to determine:

(A) Which life stage (juvenile or young adult) is to be utilized in the definitive test.

(B) The test solution concentrations for the definitive test.

(ii) The mysids should be exposed to a series of widely spaced concentrations of test substance (e.g., 1, 10, 100 mg/l, etc.), usually under static conditions.

(iii) This test should be conducted with both newly hatched juvenile (< 24 hours old) and young adult (5 to 6 days old) mysids. For each age class (juvenile or young adult), a minimum of 10 mysids should be exposed to each concentration of test substance for up to 96 hours. The exposure period may be shortened if data suitable for the purpose of the range-finding test can be obtained in less time. The age class which is most sensitive to the test substance in the range-finding test shall be utilized in the definitive test. When no apparent difference in sensitivity of the two life stages is found, juveniles shall be utilized in the definitive test. No replicates are required, and nominal concentrations of the chemical are acceptable.

(4) *Definitive test.* (i) The purpose of the definitive test is to determine the concentration-response curves and the 48- and 96-hour LC₅₀ values with the minimum amount of testing beyond the range-finding test.

(ii) The definitive test shall be conducted on the mysid life stage (juveniles or young adults) which is most sensitive to the test substance being evaluated.

(iii) A minimum of 20 mysids per concentration shall be exposed to five or more concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32, and 64 mg/l). An equal number of mysids shall be placed in two or more replicates. If solvents, solubilizing agents or emulsifiers have to be used, they shall be commonly used carriers and shall not possess a synergistic or antagonistic effect on the toxicity of the test substance. The concentration of solvent shall not exceed 0.1 ml/l. The concentration ranges shall be selected

to determine the concentration-response curves and LC₅₀ values at 48 and 96 hours.

(iv) Every test shall include controls consisting of the same dilution water, conditions, procedures, and mysids from the same population or culture container, except that none of the chemical is added.

(v) The dissolved oxygen concentration temperature, salinity, and pH shall be measured at the beginning and end of the test in each chamber.

(vi) The test duration is 96 hours. The test is unacceptable if more than 10 percent of the control organisms die or exhibit abnormal behavior during the 96 hour test period. Each test chamber should be checked for dead mysids at 24, 48, 72, and 96 hours after the beginning of the test. Concentration-response curves and 24-, 48-, 72- and 96-hour LC₅₀ values should be determined along with their 95 percent confidence limits.

(vii) In addition to death, any abnormal behavior or appearance shall also be reported.

(viii) Test organisms shall be impartially distributed among test chambers in such a manner that test results show no significant bias from the distributions. In addition, test chambers within the testing area shall be positioned in a random manner or in a way in which appropriated statistical analyses can be used to determine the variation due to placement.

(ix) The concentration of the test substance in the chambers should be measured as often as is feasible during the test. At a minimum, during static tests the concentration of test substance shall be measured at each concentration at the beginning and at the end of the test. During the flow-through test, the concentration of test substance should be measured at the beginning and end of the test and in at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system. Equal aliquots of test solution may be removed from each replicate chamber and pooled for analysis. Among replicate test chambers of a treatment concentration, the measured concentration of the test substance should not vary more than 20 percent.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Test chemical*. Deionized water should be used in making stock solutions of the test substance. Standard analytical methods should be used whenever available in performing the analyses. The analytical method used to measure the amount of test substance in a sample shall be validated before beginning the test by appropriate laboratory practices. An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interferences which cannot be systematically identified and corrected mathematically.

(ii) *Numerical*. The number of dead mysids shall be counted during each definitive test. Appropriate statistical analyses should provide a goodness-of-fit determination for the concentration-response curves. A 48- and 96-hour LC_{50} and corresponding 95 percent interval shall be calculated.

(d) *Test conditions*—(1) *Test species*—(i) *Selection*. (A) The mysid shrimp, *Mysidopsis bahia*, is the organism specified for these tests. Either juvenile (<24 hours old) or young adult (5 to 6 days old) mysids are to be used to start the test.

(B) Mysids to be used in chronic toxicity tests should originate from laboratory cultures in order to ensure the individuals are of similar age and experimental history. Mysids used for establishing laboratory cultures may be purchased commercially or collected from appropriate natural areas. Because of similarities with other mysids species, taxonomic verification should be obtained from the commercial supplier by experienced laboratory personnel or by an outside expert.

(C) Mysids used in a particular test shall be of similar age and be of normal size and appearance for their age. Mysids shall not be used for a test if they exhibit abnormal behavior or if they have been used in a previous test, either in a treatment or in a control group.

(ii) *Acclimation*. (A) Any change in the temperature and chemistry of the dilution water used for holding or culturing the test organisms to those of the test shall be gradual. Within a 24-

hour period, changes in water temperature shall not exceed 1 °C, while salinity changes shall not exceed 5 percent.

(B) During acclimation mysids should be maintained in facilities with background colors and light intensities similar to those of the testing areas.

(iii) *Care and handling*. Methods for the care and handling of mysids such as those described in paragraph (f)(1) of this section can be used during holding, culturing and testing periods.

(iv) *Feeding*. Mysids should be fed during testing. Any food utilized should support survival, growth and reproduction of the mysids. A recommended food is live *Artemia* spp. (48-hour-old nauplii).

(2) *Facilities*—(i) *Apparatus*. (A) Facilities which may be needed to perform this test include: (1) flow-through or recirculating tanks for holding and acclimating mysids; (2) a mechanism for controlling and maintaining the water temperature during the holding, acclimation and test periods; (3) apparatus for straining particulate matter, removing gas bubbles, or aerating the water, as necessary; and (4) an apparatus for providing a 14-hour light and 10-hour dark photoperiod with a 15 to 30 minute transition period. In addition, for flow-through tests, flow-through chambers and a test substance delivery system are required. Furthermore, it is recommended that mysids be held in retention chambers within test chambers to facilitate observations and eliminate loss of test organisms through outflow water. For static tests, suitable chambers for exposing test mysids to the test substance are required. Facilities should be well ventilated and free of fumes and disturbances that may affect the test organisms.

(B) Test chambers shall be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions.

(ii) *Cleaning*. Test substance delivery systems and test chambers shall be cleaned before each test following standard laboratory practices.

(iii) *Construction materials*. (A) Materials and equipment that contact test solutions should be chosen to minimize

sorption of test chemicals from dilution water and should not contain substances that can be leached into aqueous solution in quantities that can affect test results.

(B) For use in the flow-through test, retention chambers utilized for confinement of test organisms can be constructed with netting material of appropriate mesh size.

(iv) *Dilution water.* (A) Natural or artificial seawater is acceptable as dilution water if mysids will survive and successfully reproduce in it for the duration of the holding, acclimating and testing periods without showing signs of stress, such as reduced growth and fecundity. Mysids shall be cultured and tested in dilution water from the same origin.

(B) Natural seawater shall be filtered through a filter with a pore size of <20 microns prior to use in a test.

(C) Artificial seawater can be prepared by adding commercially available formulations or by adding specific amounts of reagent-grade chemicals to deionized water. Deionized water with a conductivity less than 1 $\mu\text{ohm}/\text{cm}$ at 12 °C is acceptable for making artificial seawater. When deionized water is prepared from a ground or surface water source, conductivity and total organic carbon (or chemical oxygen demand) shall be measured on each batch.

(v) *Test substance delivery system.* In flow-through tests, proportional diluters, metering pumps, or other suitable systems should be used to deliver test substance to the test chambers. The system used shall be calibrated before each test. Calibration includes determining the flow rate through each chamber and the concentration of the test substance in each chamber. The general operation of the test substance delivery system should be checked twice daily during a test. The 24-hour flow through a test chamber shall be equal to at least 5 times the volume of the test chamber. During a test, the flow rates should not vary more than 10 percent among test chambers or across time.

(3) *Test parameters.* Environmental parameters of the water contained in test chambers shall be maintained as specified below:

(i) The test temperature shall be 25°C. Excursions from the test temperature shall be not greater than $\pm 2^\circ\text{C}$.

(ii) Dissolved oxygen concentration between 60 and 105 percent saturation. Aeration, if needed to achieve this level, shall be done before the addition of the test substance. All treatment and control chambers shall be given the same aeration treatment.

(iii) The number of mysids placed in a test solution shall not be so great as to affect results of the test. Loading shall not exceed 30 mysids per liter for a static test. Loading requirements for the flow-through test will vary depending on the flow rate of dilution water. The loading shall not cause the dissolved oxygen concentration to fall below the recommended levels.

(iv) Photoperiod of 14 hours light and 10 hours darkness, with a 15 to 30 minute transition period.

(v) Salinity of 20 parts per thousand ± 3 percent.

(e) *Reporting.* The sponsor shall submit to the EPA all data developed during the test that are suggestive or predictive of acute toxicity and all concomitant toxicologic manifestations. In addition to the general reporting requirements prescribed in part 792—*Good Laboratory Practice Standards* of this chapter, the reporting of test data shall include the following:

(1) The source of the dilution water, its chemical characteristics (e.g., salinity, pH, etc.) and a description of any pretreatment.

(2) Detailed information about the test organisms, including the scientific name and method of verification, age, source, history, abnormal behavior, acclimation procedures and food used.

(3) A description of the test chambers, the depth and volume of solution in the chamber, the way the test was begun (e.g., conditioning, test substance additions, etc.), the number of organisms per treatment, the number of replicates, the loading, the lighting, the test substance delivery system and the flow rate expressed as volume additions per 24 hours.

(4) The measured concentration of test substance in test chambers at the times designated.

(5) The number and percentage of organisms that died or showed any other

adverse effects in the control and in each treatment at each observation period.

(6) Concentration-response curves shall be fitted to mortality data collected at 24, 48, 72, and 96 hours. A statistical test of goodness-of-fit shall be performed and the results reported.

(7) The 96-hour LC_{50} and when sufficient data have been generated, the 24-, 48-, and 72-hour LC_{50} 's and the corresponding 95-percent confidence limits and the methods used to calculate the values. These calculations shall be made using the average measured concentration of the test substance.

(8) Methods and data records of all chemical analyses of water quality and test substance concentrations, including method validations and reagent blanks.

(9) The data records of the holding, acclimation and test temperature and salinity.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) U.S. Environmental Protection Agency, "Bioassay Procedures for the Ocean Disposal Permit Program," EPA Report No. 600-9-78-010 (Gulf Breeze, Florida, 1978).

(2) [Reserved]

[50 FR 39321, Sept. 27, 1985, as amended at 52 FR 19068, May 20, 1987; 52 FR 26150, July 13, 1987]

§ 797.1950 Mysid shrimp chronic toxicity test.

(a) *Purpose.* This guideline is intended for use in developing data on the chronic toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003, 15 U.S.C. 2601 *et seq.*). This guideline prescribes tests using mysids as test organisms to develop data on the chronic toxicity of chemicals. The United States Environmental Protection Agency (EPA) will use data from these tests in assessing the hazard of a chemical to the aquatic environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and in part 792—*Good Laboratory Practice Standards* of this chap-

ter apply to this test guideline. The following definitions also apply to this guideline:

(1) "Chronic toxicity test" means a method used to determine the concentration of a substance that produces an adverse effect from prolonged exposure of an organism to that substance. In this test, mortality, number of young per female and growth are used as measures of chronic toxicity.

(2) "Death" means the lack of reaction of a test organism to gentle prodding.

(3) "Flow-through" means a continuous or an intermittent passage of test solution or dilution water through a test chamber or a holding or acclimation tank, with no recycling.

(4) "G1 (Generation 1)" means those mysids which are used to begin the test, also referred to as adults; G2 (Generation 2) are the young produced by G1.

(5) " LC_{50} " means that experimentally derived concentration of test substance that is calculated to kill 50 percent of a test population during continuous exposure over a specified period of time.

(6) "Loading" means the ratio of test organism biomass (gram, wet weight) to the volume (liters) of test solution in a test chamber.

(7) "MATC" (Maximum Acceptable Toxicant Concentration) means the maximum concentration at which a chemical can be present and not be toxic to the test organism.

(8) "Retention chamber" means a structure within a flow-through test chamber which confines the test organisms, facilitating observation of test organisms and eliminating washout from test chambers.

(c) *Test procedures*—(1) *Summary of the test.* (i) In preparation for the test, the flow of test solution through each chamber is adjusted to the rate desired. The test substance is introduced into each test chamber. The rate at which the test substance is added is adjusted to establish and maintain the desired concentration of test substance in each test chamber. The test is started by randomly introducing mysids acclimated in accordance with the test design into retention chambers within the test and the control chambers.